REMARKS

Status of the Claims

Currently, claims 17, 18, 38-40 and 43-47 are pending. In the Office Action, the Examiner states that all prior rejections have been withdrawn (namely: (1) the rejection of claim 41 under 35 U.S.C. Section 112, second paragraph; (2) the rejection of claim 41 under 35 U.S.C. Section 101; (c) the rejection of claims 38-40 and 43 under 35 U.S.C. Section 102(b) as being anticipated by Evans et al.; (d) the rejection of claim 41 under 35 U.S.C. Section 103(a) as being obvious over Evans et al. in view of Van Ness et al.; and (e) the rejection of claims 17-18 under 35 U.S.C. Section 103(a) as being obvious over Evans et al. in view of Van Ness et al. and Wittwer et al.). Applicants thank the Examiner for removing each of the above rejections.

In the present Office Action, the Examiner has rejected the claims on new grounds which will be addressed in more detail herein.

Rejection of Claims 38-40 and 43-45 Under 35 U.S.C. Section 102(b)

Claims 38-40 and 43-45 are rejected under 35 U.S.C. Section 102(b) as being anticipated by Steen et al., *Pharmacogenetics,* 5:215-225 (1995)) (hereinafter "Steen et al."). According to the Examiner, Steen et al. teach the method of claim 38. Specifically, the Examiner says that Steen et al. teach a method for detecting a target nucleic acid sequence suspected of having a deletion of at least 50 base pairs (a 13 kb gene deletion) in a test sample that involves the steps of:

(a) contacting the test sample with amplification reagents comprising amplification primer (the Examiner refers to page 217, col. 2, paragraph 1, under "PCR-based gene deletion assay");

(b) subjecting the reaction mixture to amplification conditions to form a target nucleic acid sequence amplification product and a standard nucleic acid amplification product (the Examiner refers to page 217, col. 2, paragraph 1, under "PCR-based gene deletion assay");

(c and d) detecting a first and second signal corresponding to a deletion and a standard nucleic acid (a 13 kb deletion and a standard 3.5 kb nucleic acid) (the Examiner refers to page 220, Figure 3); and

(e) comparing the first and second signals to determine a deletion or insertion of at least 50 base pairs is present in the DNA in the test sample (the Examiner refers to page 220, Fig. 3, col. 1, lines 4-13 of paragraph 1, column 2, paragraphs 1-2), wherein the amplification reagents comprise one primer that hybridizes to both the target and standard nucleic acid sequence (the Examiner refers to page 220, Fig. 3, where the legend states that the primers CYP-13 and CYP-24 were designed to amplify a 3.5 kb product in the presence of the 13 kb gene deletion allele indicating that the primers in the amplification reagent hybridize to both the target deletion and the standard nucleic acid sequence).

With respect to claims 39-40, the Examiner says that Steen et al. teach that the deletion is 13 kb (the Examiner refers to page 215, abstract, page 220, Figure 3, and column 1, paragraph 1).

With respect to claim 43, the Examiner says that Steen et al. teach that the deletion is in the CYP2D6 locus which is polymorphic (the Examiner refers to page 216, col. 1, paragraphs 1-2 and column 2, paragraphs 1-2).

With respect to claim 44, the Examiner says that Steen et al. teach that the amplification reagents comprise a polymerase (the Examiner refers to page 217, column 2, paragraph 1 under "PCR-based gene deletion assay").

With respect to claim 45, the Examiner says that Steen et al. teach that the amplification conditions comprise a denaturation cycle above 90°C, an annealing temperature cycle between 45° to 65°C and raising the temperature of the reaction to a temperature sufficient to activate the polymerase (synthesis or extension cycle) (the Examiner refers to page 217, column 2, paragraph 1 under "PCR-based gene deletion assay"). Applicants respectfully traverse this rejection

Steen et al. disclose an assay that detects the D6(D) gene deletion allele by use of long-PCR technology. According to Steen et al., the D6(D) allele is the second most common poor metabolizer-associated mutation and appears as a 13 kb band on RFLP analysis using *XbaI*. The long-PCR technology used by Steen et al. is described on page 217, under the heading "PCR-based gene deletion assay". The PCR reaction described by Steen et al. employs primers that are designed to amplify a 3.5 kb PCR product that contains the D6(D) allele[1]. The thermocycling conditions employed by Steen et al. et al. were 93°C for 1 minute followed by 35 cycles of 93°C for 1 minute, 65°C for 30 seconds and 68°C for 5 minutes (See Steen et al., column 2, page 217).

Figure 3 in the first column on page 220 of Steen et al. shows the results from the PCR-based gene deletion assay described on page 217. Steen et al. employed a panel of selected DNA samples that had previously been typed by PCR and *XbaI* RFLP analysis with respect to the D6(A), D6(B), D6(D) and D6(D2) null alleles. A PCR product of approximately 3.5 kb is clearly visible in Figure 3 in lanes 2, 5, 6 and 7 corresponding to DNA samples from subjects with the CYP2D6 genotypes 29 kb/13 kb wt/D, 29 kb/13 kb B/D, 44 kb/13 kb B/D and 13 kb/13 kb D/D, respectively.

In the second column on page 220, Steen et al. describe that in order to control the sensitivity and specificity of their assay that they used the PCR-based

[1] The D6(D) allele appears as a 13 kb band on RFLP analysis using *XbaI*.

gene deletion assay on 75 different DNA samples that had already been genotyped by PCR and *XbaI* RFLP analysis to identify the D6(A), D6(B), D6(D) and D6(D2) alleles. All analyses were done blindly. The results of the PCR were then compared with previously performed Southern Analysis for detection of the 13 kb gene deletion. The comparison resulted in the 75 DNA samples being divided into three groups based on genotype: 23 samples contained the 13 kb deletion allele, 5 samples contained a rare 11 kb deletion allele and 47 samples were deletion negative.

While Steen et al. report that their assay could detect the D6(D) allele, their assay exhibited sensitivity and other problems. Specifically, Steen et al. noted that the sensitivity of their assay depended on adequate amplification of the 3.5 kb product from a DNA template with the 13 kb allele and that any technical error or failure of amplification could lead to false negative results. Therefore, Steen et al. recommend that their assay always be performed in duplicate and in the presence of a positive DNA control containing the 13 kb D6(D) allele. Moreover, Steen et al. describe an alternative procedure to reduce false negatives, namely, to multiplex a reference gene of approximately 4-5 kb size together with the 3.5 kb assay fragment. Steen et al. report that preliminary experiments performed by them to coamplify the test and internal control had thus far been unsuccessful. In fact, Steen et al. go on to state that "[S]ince long-PCR is a technically demanding method, it might be difficult to multiplex the assay. Indeed, care should be taken to avoid preferential amplification of a control fragment over the D6(D)-related product (especially with respect to the D6(B)/D6(D) genotype, see above), leading to a paradoxical problem of false negative results" (See, Steen et al., page 221, first column).

Additionally, Steen et al. state that their method should always be performed in duplicate in the presence of a positive DNA control containing the 13 kb D6(D) allele. Applicants' method does not require the use of such a

positive control. Moreover, there is no disclosure or teaching in Steen et al. of detecting a first signal that is proportional to the amount of the target nucleic acid sequence amplification product, detecting a second signal that is proportional to the amount of the standard nucleic acid amplification product and then comparing these signals to determine whether or not a deletion or insertion of at least 50 base pairs is present in the DNA in the test sample (see claim 38, steps c) – e)). Instead, the method described by Steen et al. involves detecting the D6(D) allele using a gel as shown in Figure 3. Thereupon, claim 38 is not anticipated by Steen et al. Because claims 39-40 and 43-45 depend upon claim 38[2] these claims are also not anticipated by Steen et al. Therefore, the rejection of claims 38-40 and 43-45 Under 35 U.S.C. Section 102(b) is improper and should be withdrawn.

Rejection of Claims 17-18 and 46-47 Under 35 U.S.C. Section 103(a)

Claims 17-18 and 46-47 are rejected under 35 U.S.C. Section 103(a) as being unpatentable over Steen et al. in view Wittwer et al. (U.S. Patent No. 6,232,079). First, prior to addressing the rejection of claims 17-18 and 47 as being obvious in Steen et al. and Wittwer et al., Applicants submit that the Examiner has mistakenly included claim 46 in this rejection. Claim 46 is dependent on claim 38. Claim 38 is not included in this rejection. Therefore, the rejection of claim 46 as being obvious in view of Steen et al. and Wittwer et al. is improper and should be withdrawn.

With respect to Steen et al., the Examiner repeats the rejection made above in connection with the 35 U.S.C. Section 102(b) rejection while also referring to the amplification conditions described by Steen et al. page 217, column 2, paragraph 1 under "PCR-based gene deletion assay". Additionally, the Examiner admits that Steen et al. do not teach the use of a probe in an amplification reaction.

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[2] Claim 45 is dependent upon claim 44 which is dependent upon claim 38.

With respect to Wittwer et al., the Examiner states that Wittwer et al. teach a method for monitoring hybridization during PCR for detecting a target nucleic acid sequence in a test sample, comprising:

(a) contacting the test sample with amplification reagents comprising a polymerase, a PCR primer pair, and a probe (the Examiner refers to column 6, lines 1-15, column 44, lines 24-38 of Wittwer et al.);

(b) performing PCR cycles (i) raising temperature to dissociate the double-stranded genomic DNA, (ii) lowering the temperature to allow primers and probe to hybridize to the target nucleic acid, (iii and iv) raising the temperature to dissociate the target-probe hybrids and extending the primers and continuously raising the temperature to permit temperature dependent polymerase extension (the Examiner refers to column 44, lines 50-67, column 45, lines 1-12 of Wittwer et al.);

(c) repeatedly performing the PCR cycles to form an amplification product (the Examiner refers to column 45, lines 13-53 of Wittwer et al.); and

(d) detection of the amplification product as an indication of the presence of the nucleic acid (the Examiner refers to column 45, lines 13-53).

According to the Examiner, it would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to combine the method of amplification of a target nucleic acid as taught by Steen et al. with the step of primer extension in the presence of a probe or monitoring hybridization during PCR as taught by Wittwer et al. to achieve the expected advantage of developing a sensitive and enhanced method for amplification of a specific target. According to the Examiner, one of ordinary skill in the art would have had a reasonable expectation of success that the modification of the method taught by Steen et al. with the step of monitoring hybridization during PCR would result in continuously monitoring of DNA amplification, identification

and quantitation of nucleic acid and reducing laborious processing steps after PCR to identify the target nucleic acid (the Examiner refers to column 3, lines 14-33 of Wittwer et al.) and such modification of the method is considered obvious over the cited prior art in the absence of secondary considerations. Applicants respectfully traverse this rejection.

The deficiencies of Steen et al. are discussed *supra*. Applicants' arguments are incorporated herein. Moreover, the Applicants make the following additional arguments. The method of Steen et al. does not employ any probes. Moreover, Applicants submit that the cycle steps described by Steen et al. are different than the cycle steps claimed in steps b-i) to b-iv) of claim 17 and steps i) – iv) of claim 47. Thereupon, because the method of Steen et al. does not employ probes it does not disclose or suggest maintaining the reaction mixture for a time and at a temperature at least 1°C above the temperature in step b-iii) of claim 17 and step iii) of claim 47 sufficient to dissociate the probe hybrids (in the event that the probe is not completely complementary to the nucleic acid). To further illustrate the difference between the steps of the cycle of the present invention and the steps of the cycle disclosed by Steen et al., a comparison between the steps of the two cycles is provided for below in Table 1.

Table 1

Cycle steps recited in claims 17 and 47	**Steen et al. on page 217 describes 35 cycles of:**
(i) maintaining the reaction mixture for a time and a temperature above 90°C sufficient to dissociate double stranded nucleic acid sequences;	(i) 93°C for 1 minute (note that prior to this step that the machine is allowed to come up to a temperature of 93°C for 1 minute).
(ii) maintaining the reaction mixture for a	(ii) 65°C for 30 seconds.

time and at a temperature of from about 45°C to 65°C to allow the PCR primers and probe to hybridize to the nucleic acid and thereby form primer hybrids and probe hybrids;	
(iii) maintaining the reaction mixture for a time and at a temperature at least 1°C above the temperature in (ii), sufficient to dissociate the probe hybrids, if the probe is not completely complementary to the nucleic acid; and	
(iv) raising the temperature of the reaction mixture to a temperature sufficient to activate the polymerase.[3]	(iii) 68°C for 5 minutes.
	(iv) 72°C for 10 minutes.

As mentioned above, Steen et al. do not employ any probes and therefore do not disclose or suggest a separate step b-iii) (in claim 17) or step iii) (in claim 47) in their cycle. Applicants submit that the step performed by Steen et al. at 68 °C for 5 minutes is where the temperature of the reaction mixture is raised to a temperature sufficient to activate the polymerase. Applicants submit that the reason the reaction was maintained at 72 °C for 10 minutes was to provide the polymerase with additional time to finish incomplete duplexes so that a clean band could be observed on the gel.

The deficiencies of Steen et al. are not cured by Wittwer et al. As discussed in Applicants Amendments filed on June 30, 2006 and January 13,

[3] The specification on page 6, lines 28-29 states that a temperature sufficient to activate polymerases is typically between 60°C and 90°C, but are "most typically thought to be optimally active at 72°C".

2006, Wittwer et al. in column 21, lines 22-32, disclose that amplification yields and product specification were optimal when denaturation (93 °C) and annealing (55 °C) were less than 1 second and that no advantage was found for longer denaturation or annealing times. Wittwer et al. also teach that yield increased with longer elongation times at 77 °C, but that there was little change with elongation times longer than 10-20 seconds. In column 44, lines 50-62, Wittwer et al. describe cycling conditions where denaturation was performed at 94 °C for 0 seconds, annealing was performed at 50 °C for 10 seconds and extension was performed at 72 °C for 0 seconds. This cycle was then repeated 50 times and then cooled to 45 °C.

The cycle steps described by Wittwer et al. are different than the cycle steps claimed in steps b-i) to b-iv) of claim 17 and steps i) – iv) of claim 47 in Applicants methods. The difference between the steps of each of the cycles is specifically shown below in Table 2.

Table 2

Cycle steps recited in claims 17 and 47	**Cycle steps described by Wittwer et al. in column 44, lines 50-62**
(i) maintaining the reaction mixture for a time and a temperature above 90°C sufficient to dissociate double stranded nucleic acid sequences;	(i) denaturation was performed at 94°C for 0 seconds
(ii) maintaining the reaction mixture for a time and at a temperature of from about 45°C to 65°C to allow the PCR primers and probe to hybridize to the nucleic acid and thereby form primer hybrids and probe hybrids;	(ii) annealing was performed at 50°C for 10 seconds
(iii) maintaining the reaction mixture for a time and at a temperature at least 1°C above the temperature in (ii), sufficient to dissociate the probe hybrids, if the probe is not completely complementary to the nucleic acid; and	
(iv) raising the temperature of the reaction mixture to a temperature sufficient to activate the polymerase.[4]	(iii) extension was performed at 72°C for 0 seconds.

In claim 17, in step b-iii), and in claim 47, step iii), the reaction mixture is maintained for a time and at a temperature at least 1°C above the temperature in the respective steps b-ii) and ii) sufficient to dissociate the probe hybrids if the probe is not completely complementary to the nucleic acid (such a probe would be considered to be a "mismatched" probe). Likewise, probe hybrids that are completely complementary to the nucleic acid will not dissociate (such a probe would be considered to be a "matched" probe). Once this step is completed, the temperature of the reaction mixture is raised to a temperature sufficient to

[4] The specification on page 6, lines 28-29 states that a temperature sufficient to activate polymerases is typically between 60°C and 90°C, but are "most typically thought to be optimally active at 72°C".

activate the polymerase (step b-iv in claim 17 and step iv in claim 47)) and to allow for extension.

In contrast, Wittwer et al. do not disclose or suggest a separate step b-iii) or iii) (claims 17 and 47, respectively) in their cycle. Rather, after annealing is performed at 50°C, the temperature of the reaction mixture is immediately raised to 72°C to activate the polymerase and to allow for extension. Thereupon, Wittwer et al. teach the simultaneous dissociation of a mismatched probe and polymerase activation. As shown above in Table 2, in Applicants' method, these cycle steps are separated into two separate steps that are performed under different reaction conditions (namely, at a certain temperature for a certain amount of time). Also, as discussed in Applicants Amendments filed on June 30, 2006 and January 13, 2006, the performance of each of the steps b-i) to b-iv) (claim 17) and steps i) to iv) (claim 47) has been found to improve the resolution of the method particularly when compared to the three step cycle methods of the prior art, such as Wittwer et al. (See the second and third experiments in Paragraphs 5 and 6 of the Declaration of Maria C. Gentile Under 37 C.F.R. Section 1.132 filed in the Amendment filed on January 13, 2006), a copy of which is enclosed herewith.

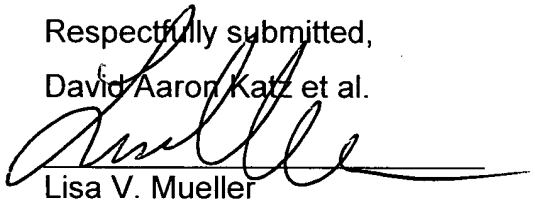
As demonstrated by the arguments above, there is absolutely nothing in Steen et al. and Wittwer et al. that disclose or suggest Applicants claimed method. Thereupon, Applicants submit that the rejection of claims 17-18 and 47 under 35 U.S.C. Section 103(a) as being obvious in view of Steen et al., and Wittwer et al. should be withdrawn.

CONCLUSION

Applicants respectfully submit that the claims comply with the requirements of 35 U.S.C. Sections 102 and 103. Accordingly, a Notice of Allowance is believed in order and is respectfully requested.

Should the Examiner have any questions concerning the above, she is respectfully requested to contact the undersigned at the telephone number listed below. If the Examiner notes any further matters which the Examiner believes may be expedited by a telephone interview, the Examiner is requested to contact the undersigned.

Respectfully submitted,
David Aaron Katz et al.



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